# Autonomic nervous system function and behavioral characteristics in (pre)adolescents from a general population cohort

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# SHORT-TERM REPRODUCIBILITY OF AUTONOMIC NERVOUS SYSTEM FUNCTION MEASURES IN 10-TO-13-YEAR-OLD CHILDREN

# CHAPTER 6

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# ABSTRACT

Despite extensive use of autonomic measures in children, short-term reproducibility of these measures is not well-known. Therefore, we investigated day-to-day reproducibility of continuous non-invasive autonomic measurements in supine and standing positions in 17 10-to-13-year-old children. Spectral measures were calculated in the low (LF, 0.07-0.14 Hz) and high frequency (HF, 0.15-0.50 Hz) band. Measures included heart rate (HR), heart rate variability (HRV-LF, HRV-HF), systolic blood pressure (SBP), blood pressure variability (BPV), and baroreflex sensitivity (BRS). Reproducibility between sessions was evaluated by test-retest correlations, coefficients of variation (CV), and Bland-Altman plots. HR related measures were moderately-to-highly reproducible ($r$=.55-.88; CV=4.5%-9.4%) and can therefore be reliably used in (pre)adolescents. SBP, BPV and BRS showed poor-to-moderate reproducibility ($r$=.35-.71; CV=10%-16%). Results caution against the use of standing-induced autonomic reactivity scores given insufficient reproducibility ($r$=-.20-.66; CV=36%-315%).

## INTRODUCTION

In children, autonomic measures of cardiac functioning, such as heart rate (HR) and heart rate variability (HRV), have been used extensively in psychophysiological research and are also applied in pediatric medicine. For example, HRV has been associated with a wide range of behavioral and emotional variables (e.g., emotional expressivity, social skills, psychopathology) (Beauchaine 2001, Ortiz & Raine 2004), as well as with cardiac diseases in children (Massin & von Bernuth 1998).

Spectral analysis of HR patterns has often been applied to gain more insight in the underlying activity of the autonomic nervous system. For instance, HRV can be studied as a function of frequency in both the low frequency (HRV-LF, generally 0.04-0.14 Hz) and the high frequency band (HRV-HF, generally 0.15-0.40 Hz). Other, less well-studied autonomic measures are blood pressure (BP) and blood pressure variability (BPV). In children, baroreflex sensitivity (BRS) has gained increased attention recently as a measure of short-term blood pressure control (Allen *et al.* 2000, Althaus *et al.* 2004, Dalla *et al.* 2006, Dietrich *et al.* 2006).

Studies investigating the short-term reproducibility of autonomic measures have rarely been conducted in children. A review of the sparse literature shows that some studies reported satisfactory reproducibility, while other studies indicated poor reproducibility. Alkon *et al.* (2003) found strong test-retest reliability of resting HR ($r$=.79) and HRV-HF ($r$=.74) in 11 four-to-eight-year-old children. Also, Doussard-Roosevelt *et al.* (2003) demonstrated moderate week-to-week stability of HR ($r$=.48) and HRV-HF ($r$=.58) in 30 five-to-six-year-old children, based on mean correlations across three measurements in sitting position within a period of four weeks. In addition, intraclass correlations of HR ($r$=.51 to .78) and systolic blood pressure (SBP, $r$=.57 to .78) between two sessions within one- to two-weeks in 20 seven-to-nine-year-old girls appeared to be moderately high in Turley's study (2005). Furthermore, we know of one study on the reproducibility of BRS measured in the supine position in 20 children and adolescents, indicating good within-session reproducibility [coefficient of variation (CV) 21% to 24%] (Rüdiger & Bald 2001).

In contrast to these promising findings, Winsley *et al.* (2003) reported weak reproducibility of HRV-LF ($r$=.14 to .37; CV 33%-142%) and HRV-HF ($r$=.26 to .76; CV 35%-143%) in 12 children aged 11-to-12 years with data that were collected on two separate days in the supine position and during light exercise. Also Tanaka *et al.* (1998) concluded poor short-term reproducibility of HRV-LF and HRV-HF in the supine position (CV 29%-31%) and of BPV in the supine and standing positions (CV 29% and 19%, respectively) in nine healthy controls with a mean age of 14.5 years.

The reproducibility of autonomic reactivity scores (Δ) to different types of stressors is also unclear in children. One study reported moderate bi-weekly test-retest correlations of Δ HR ($r$=.39) and Δ HRV-HF ($r$=.62), in response to psychological and physical stressors in 11 four-to-eight-year-old children (Alkon *et al.* 2003), whereas another study showed overall insufficient reproducibility of Δ HR ($r$=.13 to .64) and Δ HRV-HF ($r$=-.08 to .40) as a reaction to psychological stress in five-to-six-year-old children (Doussard-Roosevelt *et al.* 2003). Thus, the few studies on the short-term reproducibility of autonomic measures in children show inconclusive findings, with generally moderate reproducibility at best.

Most pediatric reproducibility studies used primarily correlational analyses when assessing reproducibility, which may hamper a proper interpretation of results (Sandercock, 2004). Correlation coefficients do not necessarily reflect agreement between test and retest measurements (Bland & Altman 1986, Hopkins 2000). In addition, correlation coefficients are sensitive to outliers, which may erroneously decrease or increase these coefficients. As another measure of reproducibility have comparison of means frequently been applied. However, this should only be regarded as a rough way of studying reproducibility, since individual scores may still vary greatly, even when mean group scores do not differ on repeated measurement occasions.

To summarize, studies on the short-term reproducibility of autonomic measures are sparse in the pediatric literature and findings are inconclusive, which may partly result from the use of rather limited statistical methods to assess reproducibility. In addition, most reproducibility studies focused on resting measures of HR and HRV. Data on the reproducibility of other autonomic indices and on indices in non-resting conditions are rare in children.

A satisfactory test-retest reliability of autonomic indices is a prerequisite for a proper interpretation of clinical and research findings. Therefore, the aim of this study was to investigate short-term reproducibility of a number of important autonomic measures in 10-to-13-year-old children, using multiple complementary statistical methods. We were specifically interested in this age range, given our large-scale studies on autonomic functioning in this age group (Dietrich *et al.* 2006, Dietrich *et al. in press*). As autonomic measures, we included HR, HRV in the low and high frequency band, SBP, and BPV and BRS in the low frequency band, based on short-term non-invasive measurements in the supine and standing positions. In addition, we calculated reactivity (Δ) scores. The orthostatic stress test is a well-known and easily applicable paradigm to measure autonomic (dys)function. Reproducibility was investigated by comparison of means, correlation coefficients, coefficients of variation, and Bland & Altman plots.

## METHOD

### Subjects

Subjects were recruited from one elementary school in the North of the Netherlands. The study's aim and procedure were explained by a research assistant in front of one classroom of 17 pupils. Then, children and their parents were each invited by letter to participate in this study. All children's parents provided written informed consent. Children could state on a form if they were not be willing to participate in this study and return this form to their teacher. Seventeen 10-to-13-year-old elementary school children [11.6+/-0.9 years; 9 (52.9 %) boys] participated in this study. Subjects were asked not to engage in strenuous physical activities 24 hours prior to the measurements. The study was approved by the National Dutch Medical Ethics Committee.

Mean height and weight of the children were 152.5+/-5.9 cm (range 144.8-164.0 cm) and 45.0+/-9.2 kg (range 31.5-61.0 kg), respectively. Mean body mass index (BMI) was 19.2+/-2.9 kg/m$^2$ (range 15.0-23.6 kg/m$^2$). Two of the children used medication for asthma, one for allergies, and one for migraine. Three of the children had a cold on the days of measurements and one child had a headache on one day, but this did not interfere with their willingness to participate in the measurements.

## Instruments

A three-lead electrocardiogram was used to register inter-beat-intervals (IBI, ms), referring to the time period between heart beats. Spontaneous fluctuations in finger blood pressure (BP) were recorded non-invasively and continuously using the Portapres device (FMS Finapres Medical Systems BV, Amsterdam, the Netherlands). BP recordings by Finapres have been validated in children by Tanaka *et al.* (1994a). Recordings were digitized (sampling rate 100 Hz, using a DAS-12 data acquisition card for notebooks, Keithley Instruments, Cleveland, Ohio, USA) and stored on hard disk for off-line analysis.

## Procedure

Most subjects were examined between 9.00 AM and noon (N=13), but some between 12.30 PM and 3.00 PM (N=4), all in a private and quiet room at school at day room temperature. At the start of the first assessment day, subject's height and weight were measured with standardized instruments. A cuff was fixed around the middle phalanx of the third finger on the non-dominant hand to measure BP while HR was registered by an electrocardiogram. While the children were in the supine position the procedure was explained to them. They were encouraged to relax and asked not to move or speak during data acquisition. Recordings did not start until circulatory readjustments of body fluid changes were completed and signals had reached a stabilized steady-state, generally within one to three minutes, in accordance with Tanaka *et al.* (1994b). Then, BP and HR signals were recorded in the supine position during an average period of 4.5 minutes, followed by the standing position during 3.3 minutes on the average, again after signals had stabilized. Breathing rate was uncontrolled. Exactly the same procedure for the autonomic measures was pursued on the next day, at approximately the same time of day.

## Calculation of autonomic variables

HR in beats per minute (bpm) was calculated as 60,000 divided by mean IBI (ms). HRV and BPV power, and BRS were based on power spectral analysis using the transfer function technique as previously described (Dietrich *et al.* 2006, Robbe *et al.* 1987). The CARSPAN software program allows for discrete Fourier transformation of non-equidistant SBP and IBI-series. The analyzed time series were visually checked for stationarity and corrected for artifacts. The power of BPV ($mmHg^2$) and HRV-LF ($ms^2$) were described in the low frequency band (LF, 0.07-0.14 Hz). HRV-HF ($ms^2$) was defined as the high-frequency (HF) power in the 0.15-0.50 Hz band. BRS (ms/mmHg) reflects beat-to-beat HR changes caused by BP changes and was calculated as the mean modulus between IBI and SBP in the 0.07-0.14 Hz frequency band with a coherence of more than 0.3. We have previously shown that coherence levels of 0.3 and 0.5 yield highly similar BRS values (Dietrich *et al.* 2006). It has been demonstrated that the narrow band around 0.10 Hz is a valid band for determining changes in short-term BP regulation and that the frequency range above 0.07 Hz is sufficient for the determination of BRS, since coherence may be insufficient in lower frequency ranges (Robbe *et al.* 1987, van Roon *et al.* 2004). Furthermore, we set a minimal limit of 100 successive seconds of acceptable signal recordings for calculation of spectral measures. In our previous study, we demonstrated the reliability of 100-second-intervals compared to 200-second-intervals (Dietrich *et al.* 2006).

## Statistical Analysis

To approximate a normal distribution, we transformed BPV, HRV-LF, HRV-HF, and BRS values to their natural logarithm before entering them in statistical analyses. To facilitate comparability of BRS values with those presented in the literature, we also present untransformed values of BRS. Reactivity ($\Delta$) scores were calculated by subtracting values measured in the supine position from those in the standing position. Posture effects (i.e., supine versus standing) of the autonomic measures were investigated by Student's paired t-tests.

To indicate reproducibility, group means of all autonomic variables (supine, standing, $\Delta$) derived from both test and retest sessions were first compared by Student's paired t-tests. Second, Pearson's correlation coefficients were used to compute test-retest correlations, which provide information on the strength of the relationship (we additionally calculated Spearman's rho to reduce the possible influence of extreme values on correlation coefficients). A correlation between 0.8-1.0 is usually considered as high, between 0.5-0.7 as moderate, and lower than 0.4 as poor, although limits are arbitrary. The probability level for statistical significance was set at 0.05.

Third, coefficients of variation (CV) were calculated for each autonomic variable (supine, standing, Δ) by dividing the group mean of the within-subjects standard deviation (SD) by the group mean of the average autonomic values of both measurement days [$SD_{within\text{-}subjects}/M_{(day1\ +\ day2)/2}$ * 100]. Although arbitrary, a CV of less than 10% is generally regarded as reflecting high reproducibility, of around 15% as moderate (see also Iellamo *et al.* 1996).

Finally, we presented Bland & Altman plots to estimate agreement between both measurement sessions (Bland & Altman 1986; R Development Core Team 2006). The Bland & Altman plot is a graphical method in which differences within each pair of repeated measurements on single subjects [day 2 – day 1] are plotted against the average of the two measurements [(day 1 + day 2)/2]. A horizontal line represents the mean difference between the repeated measurements and is expected to be zero, since the same method was used. Limits of agreement define the range within which 95% of the differences between two measurements will fall. Whether the limits are acceptable is largely a matter of judgment and depends on the purpose for which the measurements are used. Also, the plot may be used to check for bias in difference scores that may vary systematically over the range of mean values.

Reproducibility of values should at least be moderate. We define sufficient reliability as follows: (1) no significant differences in group means across measurements, (2) test-retest correlations > .5, (3) CV < 15%, (4) Bland & Altman plots show mean differences close to zero and the range of the limits of agreement can be considered acceptable.

## RESULTS

### Availability of autonomic variables

Of 408 possible observations across all autonomic variables (i.e., HR, HRV-LF, HRV-HF, SBP, BPV, BRS) for the 17 subjects who were measured in the supine and standing positions on two consecutive days (6 variables x 17 subjects x 2 positions x 2 days), 36 (8.8%) were missing. Reasons included failure of signal registration (12 observations), recordings consisting of less than 100 successive seconds of reliable signals (6 observations), observations containing more than 10% of interpolations of BP values and/or too many artifacts (e.g., showing signal gaps of more than 10 seconds of SBP signals and/or more than 5 seconds of IBI's; no extra-systoles were found) (15 observations), and observations based on fewer than three frequency points in the low frequency range (3 observations).

### Description of autonomic variables

Table 1 shows descriptive statistics of the autonomic variables measured in the supine and standing positions, and of Δ scores on two consecutive days. On both days, HR, SBP, and BPV were significantly higher in the standing than in the supine position, whereas HRV-HF and BRS were lower. HRV-LF did not significantly differ between postures.

## Reproducibility

### Comparison of means

Mean values of all autonomic variables measured in both the supine and standing positions on day 1 did not differ significantly from mean values on day 2, indicating comparable mean values on test and retest sessions (table 1). Of note, between-day differences in HRV-LF ($t$=-1.9, $p$=0.072) and SBP ($t$=2.0, $p$=0.069) measured in the standing position approached significance, i.e., showing a trend for non-reproducible mean values. With the exception of Δ SBP ($t$=4.1, $p$=0.002), all mean Δ scores of day 1 did not differ significantly from day 2. Thus, overall results generally indicate no significant differences in mean values from test to retest sessions for autonomic variables in the supine and standing positions and for Δ scores.

### Test-retest correlations

Table 2 describes test-retest correlations between autonomic variables measured on two consecutive days, in both the supine and standing positions and of Δ scores. In the supine position, all HR variables (i.e., HR, HRV-LF, HRV-HF) demonstrated statistically significant moderate-to-high test-retest correlations ($r$=.55 to .88), whereas correlations regarding SBP, BPV, and BRS were poor-to-moderate, but non-significant in the supine position ($r$=.35 to .45). Additional analyses with Spearman's rho showed a statistically significant moderate correlation coefficient for supine BRS; all other results were nearly identical. In the standing position, all autonomic variables showed significant moderately high test-retest correlations ($r$=.55 to .72). However, Δ scores yielded only non-significant test-retest correlations which were poor-to-moderate ($r$=-.20 to .45), with the only exception of Δ HRV-HF ($r$=.66, $p$=0.007). Also, Δ HR showed a trend for significance ($r$=.66, $p$=0.096). Thus, overall, test-retest correlations appeared satisfactory, except supine BP variables and most Δ scores.

**Table 1.** Means, SD, and ranges of autonomic variables measured in the supine and standing positions, and of Δ scores (standing minus supine) on two consecutive days.

| | Supine day 1 | Standing day 1 | Δ day 1 | Supine day 2 | Standing day 2 | Δ day 2 |
|---|---|---|---|---|---|---|
| | Mean (SD) (Range) | Mean (SD) (Range) | Mean (SD) (Range) | Mean (SD) (Range) | Mean (SD) (Range) | Mean (SD) (Range) |
| **HR** | 71.3 (7.26) | 92.7 (9.3) | 21.4 (8.4) | 70.3 (8.8) | 94.0 (15.0) | 23.7 (11.4) |
| (bpm) | (54.3–80.9) | (76.5–108.3) | (9.2–39.6) | (56.8–85.7) | (68.9–132.6) | (8.6–54.0) |
| **HRV-LF** | 7.0 (1.0) | 7.1 (0.7) | 0.1 (1.1) | 7.2 (0.8) | 7.5 (0.7) | 0.3 (0.8) |
| ln($ms^2$) | (4.3–8.3) | (5.6–8.2) | (-2.0–2.1) | (5.9–8.6) | (6.6–8.7) | (-1.5–1.8) |
| **HRV-HF** | 7.9 (1.1) | 6.4 (0.8) | -1.5 (1.1) | 8.0 (1.1) | 6.7 (1.0) | -1.3 (0.7) |
| ln($ms^2$) | (5.9–9.8) | (5.1–7.9) | (-4.3–-0.3) | (5.6–9.3) | (4.8–8.2) | (-2.3–-0.3) |
| **SBP** | 97.7 (18.9) | 117.4 (15.9) | 19.7 (14.8) | 93.4 (16.7) | 127.1 (22.9) | 33.7 (12.1) |
| (mmHg) | (73.4–130.2) | (87.6–142.1) | (-5.4–48.6) | (68.1–121.4) | (88.5–162.6) | (11.4–54.3) |
| **BPV** | 4.5 (0.6) | 4.9 (0.7) | 0.4 (0.8) | 4.7 (0.7) | 5.3 (0.8) | 0.6 (0.8) |
| ln($mmHg^2$) | (3.7–5.5) | (3.7–6.1) | (-0.5–2.5) | (3.6–6.1) | (3.8–6.5) | (-1.1–2.2) |
| **BRS** | 2.6 (0.4) | 1.9 (0.3) | -0.7 (0.5) | 2.5 (0.5) | 1.8 (0.5) | -0.7 (0.5) |
| ln(ms/mmHg) | (1.7–3.2) | (1.5–2.5) | (-1.7–-0.1) | (1.6–3.1) | (0.8–2.5) | (-1.7–-0.1) |
| (ms/mmHg) | 14.4 (5.1) | 6.7 (2.1) | -7.7 (5.8) | 12.9 (5.6) | 7.0 (2.9) | -6.0 (4.9) |
| | (5.4–25.1) | (4.4–11.7) | (-20.7–-0.4) | (5.1–21.1) | (2.3–11.7) | (-14.5–0.6) |

***Notes:*** HR=heart rate; HRV-LF=low frequency heart rate variability (0.07-0.14 Hz); ln=natural logarithm; HRV-HF=high frequency heart rate variability (0.15-0.50 Hz); SBP=systolic blood pressure; BPV=blood pressure variability (0.07-0.14 Hz); BRS=baroreflex sensitivity (0.07-0.14 Hz); Δ=Standing minus supine values; N=9 to 16; Supine versus standing: all significant at $p<0.001$, except BPV day 2 $p<0.05$ and HRV-LF on both days non-significant. Day 1 versus day 2: all non-significant, except Δ SBP ($p=0.002$).

### Coefficients of variation (CV)

CVs are given in table 2. The lowest CVs (indicating best reproducibility) were found for all HR variables (i.e., HR, HRV-LF, HRV-HF) in both the supine and standing positions (4.5% to 9.4%), with slightly lower CVs for HR and HRV-HF in the supine than the standing position. This points to high overall reproducibility. In contrast, CVs of SBP, BPV, and BRS were about twice as high as the CVs of HR variables in both the supine and standing positions (10% to 16%), which still indicates moderate reproducibility. However, CVs of Δ scores demonstrated poor-to-insufficient reproducibility (36% to 315%).

**Table 2.** Test-retest correlations (Pearson's *r*) between autonomic variables measured on day 1 versus day 2, and coefficients of variation (CV) of variables measured in the supine and standing positions, and of Δ scores.

| | Supine | | | Standing | | | Δ | | |
|---|---|---|---|---|---|---|---|---|---|
| | *r* | *p* | *CV* (%) | *r* | *p* | *CV* (%) | *r* | *p* | *CV* (%) |
| HR (bpm) | **.69** | .003 | 6.3% | **.55** | .034 | 9.4% | .45 | .096 | 36.2% |
| HRV-LF ln(ms$^2$) | **.55** | .029 | 8.9% | **.66** | .008 | 6.2% | .30 | .269 | 315.3% |
| HRV-HF ln(ms$^2$) | **.88** | .001 | 4.5% | **.72** | .002 | 7.7% | **.66** | .007 | 44.6% |
| SBP (mmHg) | .45 | .090 | 13.8% | **.69** | .008 | 10.0% | .43 | .158 | 59.2% |
| BPV ln(mmHg$^2$) | .35 | .208 | 16.1% | **.62** | .024 | 11.2% | .07 | .805 | 141.5% |
| BRS ln(ms/mmHg) | .35 | .264[1] | 12.6% | **.71** | .007 | 14.9% | -.20 | .605 | 75.3% |

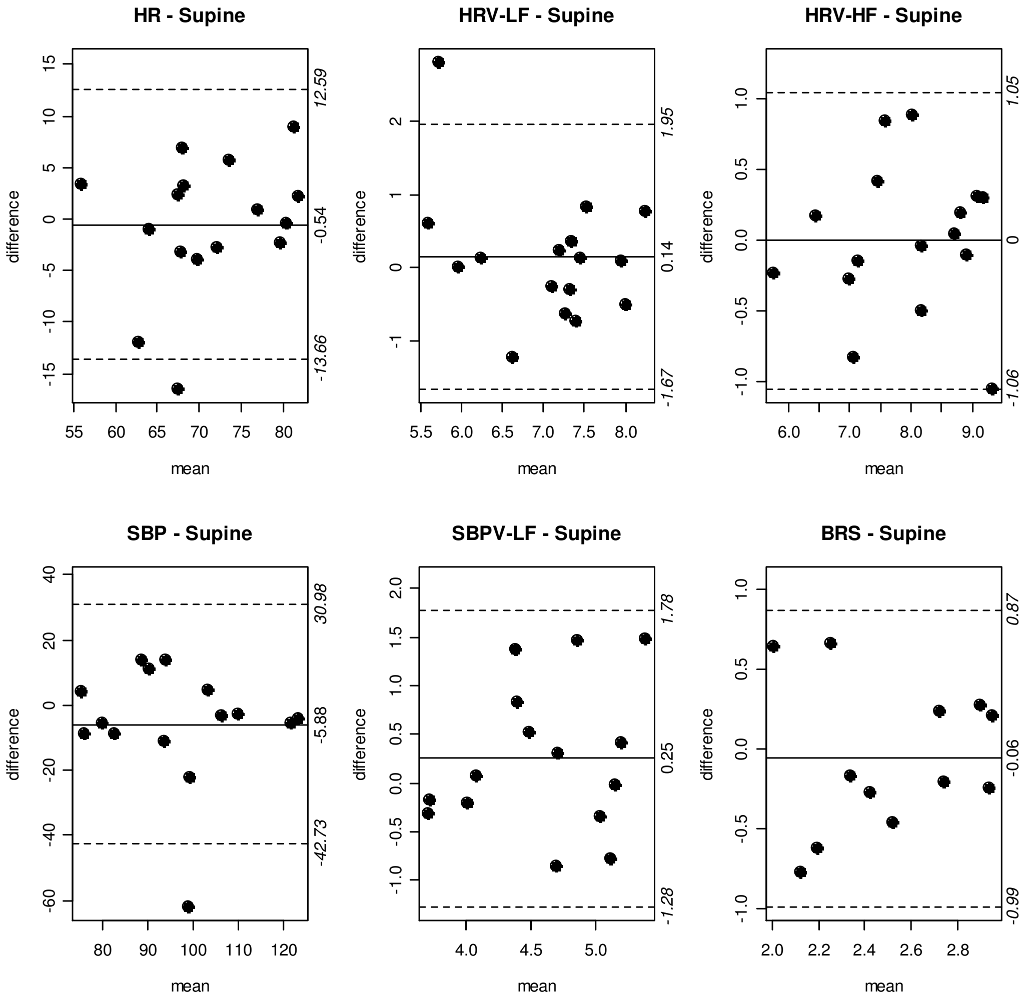
***Notes:*** HR=heart rate; HRV-LF=low frequency heart rate variability (0.07-0.14 Hz); ln=natural logarithm; HRV-HF=high frequency heart rate variability (0.15-0.50 Hz); SBP=systolic blood pressure; BPV=blood pressure variability (0.07-0.14 Hz); BRS=baroreflex sensitivity (0.07-0.14 Hz); Δ=Standing minus supine values; Supine and standing N=13 to 16, Δ N=9 to 15; [1]Spearman's rho=.59, *p*=0.042. Significant correlations are presented in bold *p*<0.05.

### Bland & Altman plots

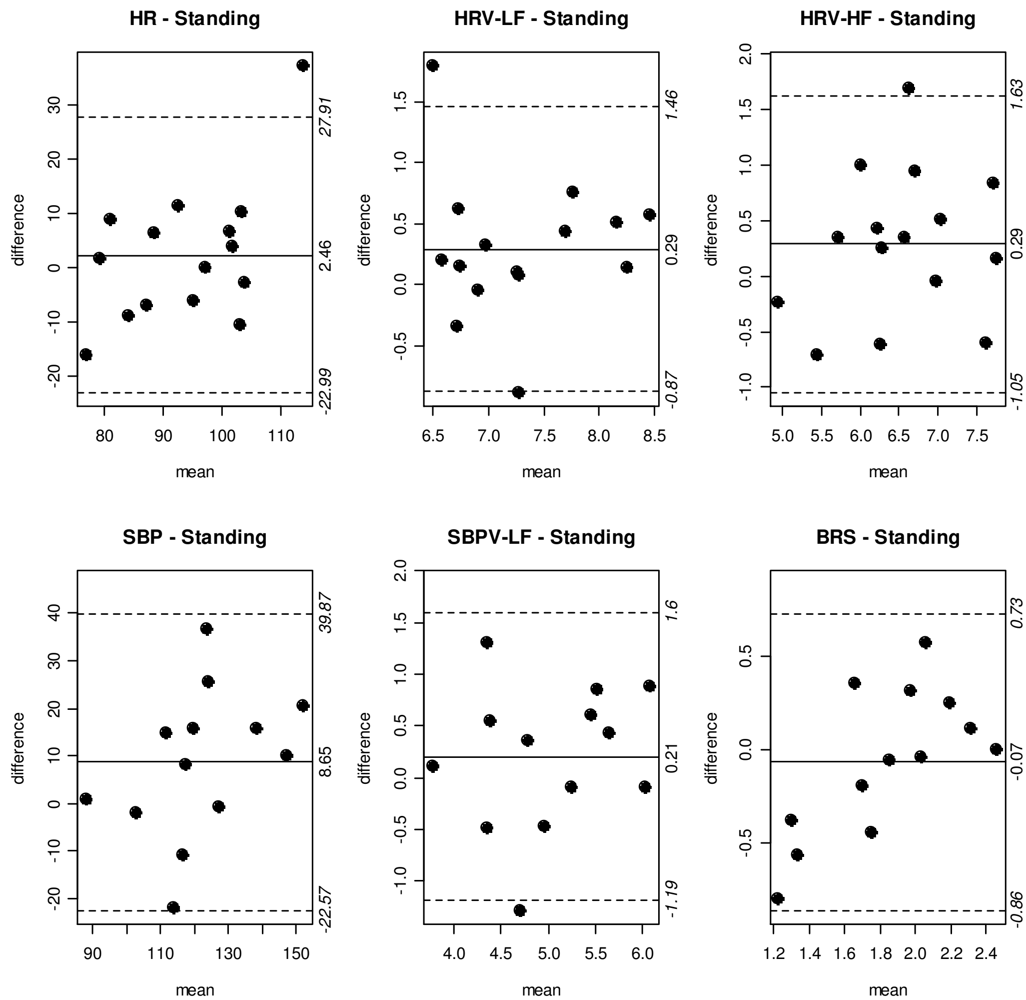
Figure 1 presents Bland & Altman plots for all autonomic measures in both the supine and standing positions, and for Δ scores. The plots demonstrate that the mean difference is close to zero for most variables, indicating satisfactory reproducibility (only the mean difference of supine and standing SBP and Δ HR deviate slightly from zero). Limits of agreement are higher for HR and HRV-HF in the standing than in the supine position, thus reflecting a higher degree of variability of difference scores in the standing position. Conversely, the limits of agreement of SBP, BPV, and BRS appear lower in the standing than in the supine position, suggesting lower variability in the standing position. Some bias appears to exist regarding BRS in both positions; across the range of lower BRS values difference scores appear larger and more variable than across the range of higher BRS. Overall, when considering absolute ranges of limits of agreement, all variables measured in the supine and standing positions are satisfactorily reproducible. In contrast, ranges of limits of agreement of Δ scores appeared to be unacceptably high.

**Figure 1.** Bland & Altman plots for heart rate (HR), heart rate variability in the low (HRV-LF) and high frequency band (HRV-HF), systolic blood pressure (SBP), blood pressure variability (BPV), and baroreflex sensitivity (BRS) (a) in supine position, (b) in standing position, and (c) regarding Δ (standing minus supine) scores. HRV, BPV, and BRS are ln-transformed. Mean=average scores of both measurements [(day 1 + day 2)/2]. Difference=difference within each pair of measurement [day 2 – day 1]. The bold line and number attached to it shows the mean difference score. Dashed lines and numbers attached to it represent the mean difference +/- 1.96 SD, or 95% limits of agreement.

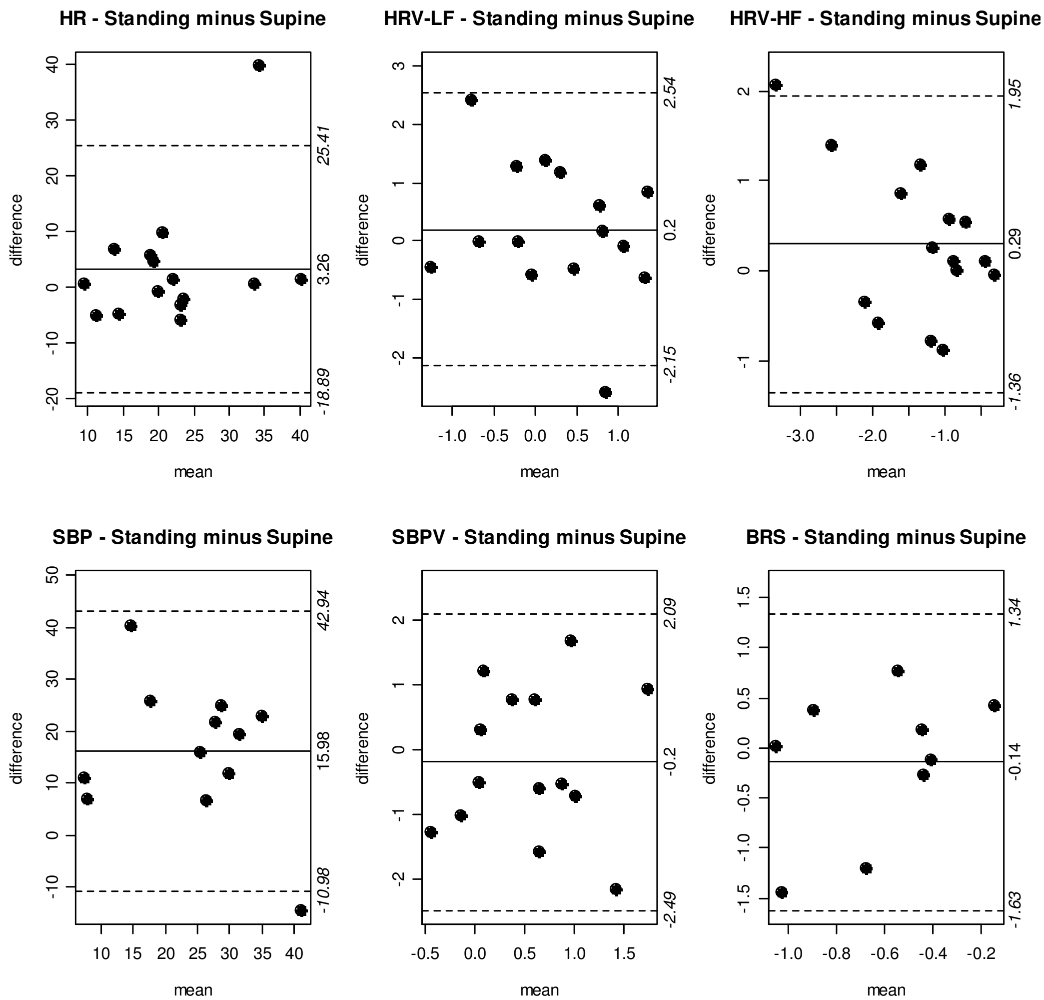
(a)


HR - Supine
difference
mean
12.59
-0.54
-13.66
HRV-LF - Supine
difference
mean
1.95
0.14
-1.67
HRV-HF - Supine
difference
mean
1.05
0
-1.06
SBP - Supine
difference
mean
30.98
-5.88
-42.73
SBPV-LF - Supine
difference
mean
1.78
0.25
-1.28
BRS - Supine
difference
mean
0.87
-0.06
-0.99

(b)


HR - Standing
HRV-LF - Standing
HRV-HF - Standing
SBP - Standing
SBPV-LF - Standing
BRS - Standing
difference
mean
27.91
2.46
-22.99
1.46
0.29
-0.87
1.63
0.29
-1.05
39.87
8.65
-22.57
1.6
0.21
-1.19
0.73
-0.07
-0.86

(c)


HR - Standing minus Supine
HRV-LF - Standing minus Supine
HRV-HF - Standing minus Supine
SBP - Standing minus Supine
SBPV - Standing minus Supine
BRS - Standing minus Supine
difference
mean
25.41
3.26
-18.89
2.54
0.2
-2.15
1.95
0.29
-1.36
42.94
15.98
-10.98
2.09
-0.2
-2.49
1.34
-0.14
-1.63

# DISCUSSION

Overall, the present study indicates sufficient day-to-day reproducibility of the time domain measures HR and SBP, and the spectral measures HRV-LF, HRV-HF, BPV, and BRS. The degree of test-retest reproducibility varied considerably between the different autonomic measures, with heart rate variables generally demonstrating larger reproducibility than measures related to blood pressure and baroreflex function. Moreover, posture (i.e., supine versus standing) appeared to influence the level of reproducibility. Finally, an important finding of this study is the insufficient reproducibility of standing-induced reactivity or Δ scores (i.e., calculated as standing minus supine values).

## Reproducibility of variables related to heart rate

Reproducibility of HR in the supine and standing positions was moderate-to-high according to the magnitude of CVs and Bland & Altman plots, with correlation coefficients remarkably similar to two previous studies on HR reproducibility in children. Turley (2005) found moderately high intraclass correlations of HR ($r$=.51 to .78) between two sessions within one week in 20 seven-to-nine-year-old girls and Doussard-Roosevelt *et al.* (2003) a HR test-retest correlation coefficient of $r$=.48 in 30 five-to-six-year-olds. In addition, Alkon *et al.* (2003) reported strong test-retest reliability of resting HR ($r$=.79) in 11 four-to-eight-year-old children.

HRV-HF yielded the best reproducibility in our study, being highly reproducible in both the supine and standing positions. This is in accordance with previous adult studies, which also reported better reproducibility of autonomic measures in the HF than the LF band (Lobnig *et al.* 2003; see Sandercock *et al.* 2005). In children, also Alkon *et al.* (2003) provided evidence of good stability of HRV-HF ($r$=.74) and Doussard-Roosevelt *et al.* (2003) reported at least moderate stability of HRV-HF ($r$=.58). In contrast, Winsley *et al.* (2003) found insufficient reproducibility of HRV in both the LF-band ($r$=.14 to .37; CV 33% to 142%) and HF-band ($r$=.26 to .76; CV 35% to 143%) in 12 children aged 11-to-12 years. Also, Tanaka *et al.* (1998) concluded poor short-term reproducibility of HRV-LF and HRV-HF in the supine position (CV 31% and 29%, respectively) in nine healthy controls with a mean age of 14.5 years. However, the sample size of the latter two studies was quite small. Excessive exercise on the days before data collection may have contributed to the more pronounced day-to-day variations in previous studies in comparison to the present study (Winsley *et al.* 2003).

The excellent reproducibility of HRV-HF compared to the other autonomic measures might be understood by the fact that it is a specific index of parasympathetic activity in comparison to the more complex and integrated autonomic measures that reflect both parasympathetic and sympathetic activity (e.g., HRV-LF, BRS) (Alkon *et al.* 2003).

Interestingly, reproducibility of measurements related to heart rate appeared to be generally better in the supine than in the standing position in our study. This is in line with the conclusions of the review of Sandercock *et al.* (2005) on the reliability of HRV, which generally turned out to be worse in stimulated (e.g., during tilt) than in resting conditions. This finding might be understood by the fact that vagal activity predominates in resting situations and that HRV (in the high frequency band) is primarily an index of vagal activity. It is plausible that increased sympathetic activity during orthostatic challenge introduces noise and variability to this vagal index, making it less reproducible in a non-resting situation. Conversely, HRV in the low frequency band showed better reproducibility in the standing than in the supine position. The low frequency band is known also to be influenced by sympathetic activity. Measures of the low frequency band (as well as BP, which is largely sympathetically driven) might thus be more sensitive to sympathetic influences which predominate in the standing position and therefore be better reproducible in a non-resting condition.

## Reproducibility of blood pressure variables and baroreflex sensitivity

Measures related to blood pressure (i.e., SBP, BPV) and baroreflex function showed poor-to-moderate reproducibility in the supine position and moderate reproducibility in the standing position. Thus, these measures show better reproducibility in the standing than the supine position, which is in line with other studies regarding the reliability of supine (CV 29%) versus standing (CV 19%) BPV in children (Tanaka *et al.* 1998) and of supine versus standing BRS in adults (Iellamo *et al.* 1996, Herpin & Ragot 1997). Such findings may appear somewhat counterintuitive, since it is known that recordings in the standing position may be accompanied by a decrease in stationarity (e.g., by increased muscle activity or other disturbing movements) compared to supine measurements, probably introducing measurement error which may negatively affect reliability of recordings. Again, an explanation for the present posture effect might be found in the predominance of sympathetic activity during orthostatic challenge (Chapleau 2005).

The lower level of reproducibility of blood pressure variables and BRS compared to heart rate variables in this study may on one hand reflect larger intrinsic biological variation of measures related to blood pressure, but may also result from measurement-related errors. The larger variability in SBP scores derived from Finapres or Portapres recordings in comparison to clinical BP assessments forms a good illustration of the potential influence of the applied measurement method (Dawson *et al.* 1997).

The moderate reproducibility of BRS can be explained by the fact that it is a compound measure of HRV and BPV in the low frequency band, both containing the variability of each of these measures. It appears that the moderate reproducibility of BRS results primarily from the increased variability in BPV and less from HRV-LF, given that HRV-LF (in contrast to BPV) was highly reproducible.

Many adult studies have also reported moderate reproducibility of BRS, with CVs of about 15% to 30% (e.g., Dawson *et al.* 1997, Gao *et al.* 2005, Hojgaard *et al.* 2005, Iellamo *et al.* 1996). To our knowledge, only one other study reported on the short-term reproducibility of supine BRS in children and adolescents (Rüdiger & Bald 2001). The authors concluded good reliability of supine BRS, although the magnitude of CVs (21% to 24%) was higher than in our study. One explanation for the generally lower CVs in our study compared to other studies may be the use of natural log-transformations on our spectral measures. By using log-transformations normally distributed variables may be obtained, which is likely to change group means and reduce variance. Indeed, in reproducibility studies, smaller CVs have been found in log-transformed or normalized data than in untransformed data (Carrasco *et al.* 2003, Winsley *et al.* 2003).

## Reproducibility of standing-induced autonomic reactivity scores

Reproducibility of standing-induced reactivity or Δ scores was insufficient in our study. The general lack of reproducible Δ scores may be best understood by the combination of relatively low absolute Δ scores (or even lack of differences between the supine and standing positions) and accumulation of variance across the multiple supine and standing measurements on which the calculation of Δ scores is based. This results in an overall magnitude of measurement variance that easily overrules Δ scores themselves, resulting in CVs of well over 100%.

We know of one other pediatric study that reported unsatisfactory Δ HR ($r$=.13 to .64) and Δ HRV-HF ($r$=-.08 to .40) values (using psychological stressors) (Doussard-Roosevelt *et al.* 2003). A previous review of adult literature had also come to the conclusion that the test-retest reliability of reactivity measurements of HR and SBP should be regarded worrisome (Manuck 1994). This obviously limits the usability of autonomic reactivity scores.

When interpreting reproducibility findings from other studies, it is also important to take into account the different statistical methods. In a study of 11 four-to-eight-year-old children, test-retest correlations of Δ HR ($r$=.39, non-significant) and Δ HRV-HF ($r$=.62, $p$<0.05) induced by various stressors (social, cognitive, emotional, and physical) were reported over a two-week period (Alkon *et al.* 2003). These were of remarkably similar magnitude to ours. Based on correlational test-retest analyses, these authors concluded that short-term reliability of reactivity scores was quite strong. However, other statistical techniques (e.g., CVs, Bland & Altman plots) to investigate reproducibility were not given, preventing further comparison to our study. This again illustrates that the conclusions drawn also largely depend on the kind of statistical analyses involved.

As another example, one adult study also concluded sufficient short-term reproducibility of changes in HRV-LF and BPV due to sympathetic stimulation induced by nitroglycering infusion and head-up-tilt in healthy young male volunteers

(Cloarec-Blanchard *et al.* 1997). This conclusion was primarily based on Bland & Altman plots between both measurements, without regarding CVs as in our study, which complicates a direct comparison to our results. In the present study, CVs of Δ scores were very high (36% to 315%), thus indicating poor-to-insufficient repeatability of Δ scores. It should also be noted that sympathetic stimulation induced by nitroglycering infusion and head-up-tilt may have different effects than orthostatic challenge.

## Summary and Conclusions

To summarize, we generally found satisfactory day-to-day reproducibility of a number of autonomic measures in 10-to-13-year-old children. HR and HRV in the low and high frequency band were the most reliable measures in this study, showing a moderate-to-high level of reproducibility in both the supine and standing positions. These measures may therefore be reliably used in children approaching adolescence. However, a different picture emerges for blood pressure variables (SBP, BPV) and BRS, which demonstrated moderate reproducibility in the standing position, but only poor-to-moderate reproducibility in the supine position. Therefore, small changes over time in SBP, BPV, and BRS, obtained from non-invasive continuous blood pressure monitoring should be interpreted cautiously. Moreover, this study's results caution against the interpretation of findings regarding autonomic reactivity (Δ) to orthostatic stress.

## Limitations and further directions

More studies on the reproducibility of autonomic measures in children of different age ranges are highly needed, also in clinical groups (Sandercock *et al.* 2005), and in response to various types of physical and psychological stressors. Future research could investigate whether the reliability of autonomic measurements, in particular of autonomic reactivity (Δ scores), may be enhanced by applying several test sessions which may be averaged in order to reduce random error (Kamarck & Lovallo 2003, Swain & Suls 1996). Also, increasing the measurement period could positively influence reliability; in the present study, assessment intervals might have been too short to reliably measure Δ scores. Another limitation of this study may have been the small sample size, especially when regarding Δ scores. Nevertheless, the present findings are of particular importance, considering the increasing number of studies employing autonomic measures in the field of psychophysiology as well as pediatrics.

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